NOTE ON THE SPAWNING OF THE HOLOTHURIAN, THYONE BRIAREUS (LESUEUR)

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Thyone briareus is fairly abundant at Woods Hole, Massachusetts, yet very little has been recorded about its spawning. The present note is based upon observations made in the laboratory in 1948.

The Breeding Season

June is the principal month in which shedding has been observed in the laboratory at Woods Hole. Pearse (1909) noted spawning from June 22nd to July 5th. Ohshima (1925) reported it from June 21st to 24th in 1921, and Just (1929) observed it repeatedly during the month of June for several years. Mead (1898) found every animal full of nearly ripe eggs or sperm on April 24th, which would suggest a season beginning earlier than June, but Just (1929) claimed that eggs obtained in April and May were unripe ovocytes; capable of responding to insemination but unable to develop. There are no other data for the early part of the breeding season. As to the latter part, Pearse's observation of shedding on July 5th is the latest specific data published for *Thyone* at Woods Hole, although Bumpus (1898) remarked that the breeding season was probably June and July and Clark (1902) stated that *Thyone* apparently bred in the summer.

A study of 314 animals was made during the second half of June, 1948. Forty-nine of these animals shed in the laboratory, and 215 which did not shed were dissected. The results are summarized in Table I. The table shows the number of animals collected on each date and their condition as determined by shedding or dissection, together with estimates of shedding capacity based on the findings in these two categories. Line 8 in the horizontal direction includes some cases of undetermined sex among spent animals. When spawning is over, the gonadal tubules are so small that a careful microscopical examination is necessary to determine the sex. Such examinations were not made in the cases indicated. The single case of undetermined sex among the partly spent animals of the June 19th group was simply the result of an oversight. It is quite easy to distinguish the sexes when partly spent animals are dissected. The gonadal tubules of the male are an opaque yellowish or orange color and are pointed at the distal end, while those of the female are more translucent, golden or mustard colored, and have blunt distal ends. The dates of collection shown in the table for the different groups of animals do not necessarily indicate the dates on which spawning occurred. In fact, owing partly to experimental conditions, sheddings usually took place on subsequent dates. Nevertheless, the sheddings are listed as of the date of collection inasmuch as they indicate, no matter when they occurred, the shedding *capacity* of animals collected at that time. For example, some animals collected on June 15th were

TABLE I *

Shedding data and estimates of shedding capacity. (Explanation of categories in text.) Since shedding occurred under various experimental conditions the numbers in line 5 do not necessarily reflect maximum shedding as it might have occurred in nature.

1. Group		1			2			3			4			5			6			8			10	
2. Date		June 15			June 19			June 21 or earlier			June 21 or earlier			June 23			June 26			June 29			July 15	
3. Number in group		24			64			35			48			36			63			20			24	
4. Sex	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂
5. Number shed	1		8	3		3	3		15	5		5	0		0	2		3	0		1	0		0
Number dissected																								
6. Full	0		0	5		0	0		0	2		0	1		0	0		0	1		0	0		0
7. Partly spent	4		5	7	1	14	1		2	0		7	0		4	7		13	0		4	0		3
8. Spent	1		0	11	4	15	2		1	7		6	15		9	21		11	6		4	10	6	5
9. Remainder estimated sheddable	1.3		3.4	.23		.26	1.8		7.8	3.8		6.0	.24		.96	.93		1.66	.25		1.25	0		0
10. Estimated spent		.3			.5			1.4			6.2			5.8			3.4			2.5			0	
11. Number of each sex estimated in group	10.9		13.1	29.1		34.9	15.9		19.1	21.8		26.2	16.3		19.6	28.6		34.3	9.1		10.9	10.9		13.1
12. Number of each sex estimated sheddable	6.3		16.4	15.2		17.3	5.8		24.8	10.8		15.5	1.3		4.96	9.9		17.7	1.3		6.3	0		3
13. Per cent of each sex estimated sheddable	57.9		125.5	52.4		49.5	36.7		129.9	49.4		59.3	7.6		25.3	34.6		51.5	13.8		57.3	0		22.9

* Note. Total number of females (shed and dissected) = 115, or 45.4 per cent females
Total number of males (shed and dissected) = 138, or 54.5 per cent males
63.5 per cent of the females studied were spent
36.9 per cent of the males studied were spent
7.8 per cent of the females studied were full of eggs
None of the males studied were full of sperm

found shedding on the 18th, others not until the 22nd, 23rd and even the 26th of June, yet all of these sheddings indicate the shedding capacity of animals collected on the 15th. Compared with specimens actually collected on the 23rd or 26th, the June 15th group shows a much higher shedding capacity. The numbers collected were meager for some of the dates studied but an analysis of the table does show, in a general way at least, the probability of obtaining embryological material at this season. It may be mentioned that no single season is necessarily typical, since there is considerable variation in weather conditions during the spring months from year to year and this is probably reflected in the environment of the *Thyone* beds. A comparison of water temperatures at Woods Hole for the years 1902 through 1906 is given by Sumner, Osburn, and Cole (1911) and the air and water temperatures of recent years are on file at the Marine Biological Laboratory and the Woods Hole Oceanographic Institute (unpublished).

It has not been found possible to determine the sex of *Thyone briareus* by external inspection. Certainly there are no obvious correlations between sex and size, color, general appearance, or behavior. Therefore it is assumed that the specimens selected for dissection represented a random sampling of the entire group and showed about the same proportions of the two sexes as would any other sampling, as, for instance, the specimens left undissected. From lines 5, 6, 7 and 8 it can be seen that a total of 115 females were found, through shedding and dissection, while there were 138 males. Obviously 253 animals are not enough for a very accurate study of the sex ratio but, for want of more, this number must be used at present. Hence, it is concluded that 45.4 per cent of the animals would be females and 54.5 per cent would be males, or a ratio of about five to six, in any group of *Thyone* collected as were the subjects of this study.

Estimates of shedding capacity within a group were obtained in the following manner. (1) Animals which actually did shed were considered sheddable as of the date collected. (2) Some animals were probably prevented from shedding by the conditions to which they were subjected. If dissection showed the gonads full of seemingly mature gametes, the animals were considered sheddable. This seemed permissible since shedding did occur in the first group collected. (3) It was found subsequently that a given animal can shed more than once, at intervals of several days. Therefore, dissected animals found to be only partly spent were also considered sheddable. (4) Some animals that did not shed were not dissected, but an estimate was made showing those that should have been able to shed. (The number not examined was multiplied by the proportion found sheddable among those that were examined.) The sum of these four categories gave the estimated number of animals able to shed, from which the percentage of shedding capacity for the group could be calculated. However, since there were more males than females and since, moreover, most groups showed more females than males to be already spent, the shedding capacity was estimated in terms of percent of each sex capable of shedding. This seemed worth while in spite of the inaccuracies bound to arise from a study of such small numbers (e.g. Group 3 shows 129.9 per cent sheddable males), because the low female shedding capacity would otherwise have been masked by the higher male activity.

Table I shows that nearly half of the females examined in the middle of June had already shed their eggs. By June 21st more than half were spent, and as early as June 23rd one group showed the exceptionally low shedding capacity of only 7.6 per cent. In the last week of June, little more than a third of the females could shed and much lower percentages might be expected, such as the 13.8 per cent noted on June 29th. By July 15th there were no females able to shed, out of a group of 24 animals examined, although three of the males could still have shed some sperm. Aside from the numerical evidence of a waning season, the quantities of mature germ cells, either shed or found by dissection, dwindled as the month progressed. It seems, then, that the guess of Bumpus about shedding in June and July in 1898 would only have proved half accurate in 1948. And while one might have expected an occasional shedding of eggs as late as July 5th, as Pearse reported in 1909, that date could not have been recommended for the purpose this year. In fact, it would not have been worth while to attempt a detailed examination of the eggs of *Thyone* from animals collected after the third week of June.

Although this study was not begun until the middle of June, there are some indications of what may have occurred earlier. One might expect a higher percentage of mature females to be capable of shedding at the height of the season than actually did shed at any time from mid-June on. Nearly half of the batch was spent as early as June 19th. As early as June 15th there were as many partially spent as there were shedding, and on the 19th and again on the 23rd, there were more partially spent than the sum of the shedding and fully ripe ones combined. Certainly the season was waning during the entire second half of June. All this suggests a peak reached before June 15th. If this is correct, then the spawning season must either have been very short or have begun before June 1st. We have Mead's (1898) observation to support the latter possibility and Just's (1929) to support the former. Until the question is settled, the most promising time for successful embryological collecting must be considered to be the first half of June or possibly the middle two weeks of this month.

Shedding

Before it begins to shed, an animal expands greatly, to perhaps twice its former length, and starts waving its tentacles gently in the water in "feeding movements," as noted by Pearse in 1909. The position of the body can vary from vertical to horizontal, attached to the bottom or the sides of its container. It needs enough free space for the extended tentacles because sudden contact with any object will usually cause contraction, which might possibly delay shedding. On the other hand, shedding is not impossible in cramped quarters and once begun, under whatever circumstances, the process can be resumed in spite of various interruptions, such as removing the animal from water, leaving it practically dry, placing it under a very bright light, rinsing it in cold tap water, and so on. However, the writhing movements of an animal out of water or in too small a container will disperse the germ cells and could injure them.

As has been mentioned above, the sexes are indistinguishable externally. In both, the genital pore lies at the tip of an inconspicuous little stalk in the mid-dorsal line, between the bases of two large tentacles. It is easily located since it occupies the point on the tentacular ring diametrically opposite the only pair of small tentacles. The genital duct connects the pore with the gonads, which lie along the dorsal body wall, about halfway between the mouth and anus. Kille (1939) has described the gonads in post-spawning animals and discusses the tubules and their contents as found in July and August. It seems as if the entire gonadal knot need not be involved with spawning each year and some tubules may remain small and undistended. On the other hand this may be simply the appearance of late-season tubules. Ohshima (1925) described irregular masses of disintegrating yolk in the ovarian tubes, concluding that they were probably eggs which had failed to be laid in the previous spawning season and were undergoing degeneration. Brownish masses were especially notable in the ovarian tubules of spent females toward the end of the present study and these too were given Ohshima's interpretation, but it was thought that they probably represented degenerating eggs of the current season. Kille suggested that some large tubules may have been lost by some of his animals seen in July and August, an impression also gained from the present study, but not actually proved.

Once when an animal was vastly expanded and undergoing the writhing movements that precede shedding, the body wall became semi-transparent and the genital duct could be seen, a straight, grayish line leading to the pore. It showed clearly even when the animal was removed from the water and held up to the light. Shedding finally did occur and the animal proved to be a female as had been predicted. After the shedding the line was gone, but later a shorter line was noticed in the same region. Since the eggs are grayish and the sperm white, it is possible that some method of sex diagnosis might be worked out, but it probably would be practical only with full, mature animals. It was not effective this season.

The germ cells are emitted in a slow, fountain-like stream, so that they rise upward for a very short distance and then fall toward the bottom of the container. Since the tentacles are waving, there is fairly rapid dispersal, especially of the sperm. Moreover, animals nearby in the same container will ingest the gametes being shed by a neighbor. A female which has been in a container with a shedding male can be partially freed of sperm by rinsing in running tap water; but since the tentacles are withdrawn during this process, all the sperm cannot be reached and some of her eggs, if shed soon, are certain to be fertilized. Many changes of sea water with intervals for feeding movements in each would cut down the possibility of sperm contamination, but not eliminate it. Probably the best way to obtain unfertilized eggs under these circumstances is to hold a slender medicine dropper directly above the genital papilla. If introduced slowly and gently this is quite feasible, although tedious. It is much easier to keep prospective shedders in separate containers. Segregation is desirable even if fertilized eggs are to be collected, since the relatively large amount of sperm, even in 6 by 9 inch battery jars, will literally smother the eggs and is to be avoided.

The duration of the shedding process varied considerably, perhaps depending upon the age, and hence size, of an animal as well as on its degree of fullness. When watched, the process was sometimes as short as 10 or 15 minutes but extended up to four and a half hours in the largest shedding witnessed. Usually it was completed within about a half hour of the time of starting. Sometimes shedding is not quite continuous even when apparently undisturbed, and will proceed intermittently with intervals of from a few minutes on up to nearly three days. These latter repeated sheddings were noted several times, in one unusual case 7 and 9 days, respectively, after the animal was collected. Repeated shedding has not yet been found in a female.

The present study confirms the statements of Ohshima (1925) and Just (1929) that eggs obtained by means other than natural shedding connot be fertilized in the laboratory. It is easy to secure large eggs simply by mincing the ovarian tubules. The eggs which fall out are about as large as shed eggs. They contain a large germinal vesicle which has never been seen to break down except under pressure, applied externally. They have not been fertilized.

Factors in Shedding

No reliable method has been found to induce shedding at the will of the investigator, but various factors which might influence the phenomenon have been examined in connection with the shedding of the 35 males and 14 females which took place during the present study. The preponderance of males shedding was much greater than might be expected on the basis of the sex ratio. Perhaps they simply shed more easily or more frequently. Perhaps their activity lasts longer during the declining season. Whatever the reason, it should be borne in mind that the factors considered were effective on males particularly. They cannot be clearly demonstrated to be shedding agents until they have been examined again, at a time when many ripe females are available.

(1) Time of Day

Ohshima (1925) found that shedding always occurred late in the afternoon of the day the animals were brought into the laboratory. Once in 1948 a number of animals did shed around 6:00 P.M. of the day they were delivered, but it happened that this batch had been collected previously and kept on the water table in the supply department for at least a day. By far the majority of sheddings watched this year took place in the evening, mostly around 8:00 and 9:00 o'clock, and some as late as 1:00 or 2:00 A.M. Of course, many of these sheddings occurred under experimental conditions and could not be attributed entirely to a natural tendency. It did seem, however, as if there might have been a rather strong natural bent toward evening shedding which persisted despite external conditions. Nevertheless, at least two animals were seen shedding in the morning, one between 9:00 and 10:00 o'clock and the other at 12:00. Therefore, this part of the day need not be ruled out as a possible time for obtaining the germ cells.

(2) Light

Ohshima was able to induce animals to shed during the day by placing them in a dim light. Returned to bright light, they ceased shedding but would continue again if replaced in subdued light. Utter darkness did not cause shedding. Presumably these observations were made during the four days when Ohshima obtained eggs. During the present study sheddings were observed on at least ten different days and under many circumstances. They were seen to take place in natural, indirect daylight, in subdued daylight and in electric light of various intensities from rather subdued to bright lamplight directed right on the animals. They were also found to have occurred in complete darkness. Several times a male which had started to shed was picked up unceremoniously and transferred to a new dish under a bright electric lamp. Soon it started to shed again. Some females shed in bright light and their performance was watched in light sufficiently bright to allow one to see it easily, but it is true that at least 8 of the 14 females studied did shed in either complete or semi-darkness. The number is too small to be significant. This diversity of findings seems to rule out subdued light as a prerequisite. Possibly Ohshima's observations can be explained in the following way.

Thyone is very sensitive to changes in light which occur suddenly, as when, as Pearse showed (1908), a shadow passes abruptly between the animal and the light source. It also reacts immediately to jarring. The reaction to both stimuli is the same: contraction and in-drawing of tentacles, enough to interrupt shedding. It may take a minute or longer before the animal will expand again after such a contraction. If the shedding time were a short one, interruptions such as these would be enough to appear to stop the process. With only four days of observations, and eggs to collect at the same time, Ohshima may have returned his specimens to the dim light before they had had time to start shedding in the other types of light investigated. On the dates of his collections, June 21st to 24th, he might also have been dealing with some animals that were partly spent and had little to shed, as in the present study.

(3) *Presence of a Male*

Finally, Ohshima found no females spawning spontaneously, but only after the emission of sperm by nearby males in the same container. This year it was inconvenient to isolate all the animals of a group because of the container requirements for animals of this size, but at least two females did start to shed while wholly isolated in separate bowls. Others, isolated as soon as shedding was noticed, would continue after isolation, and this was, incidentally, usually after a rinsing in cold tap water too. The male effect was tested conversely when a female which had shed two days earlier was placed in a jar of freshly shed sperm, in an attempt to induce further shedding. It did not occur, even though subsequent dissection showed at least some mature eggs in at least some ovarian tubes. It would be more conclusive to use a full, mature female, but as yet no way has been found to distinguish such animals. At all events, isolated females are perfectly capable of initiating shedding.

(4) *Size*

The size of these animals is very hard to determine since so much depends upon their water content at the time of examination. However, any animals known to the supply department as medium or large will probably prove satisfactory as a source of germ cells, in season. It is not always the largest in a batch that will shed first, nor is the size any criterion of the sex of an animal. Very small specimens are to be eschewed for several reasons. (a) If mature, they will have only a small quantity of germ cells. (b) They may be mature animals already spent and hence reduced in bulk by the absence of distended gonads. (c) They may be small because they have eviscerated and hence are lacking in most of their viscera, usually including the larger gonad tubules. All three types have been encountered in the present study, (a) and (b) most frequently.

(5) *Quantity of Sea Water*

Just (1929) observed spawning repeatedly among animals if kept in large quantities of sea water. This year, shedding would begin in any amount of sea water sufficient to hold the animal and allow it to expand, and once begun, several males were found able to continue shedding even when placed in practically dry finger bowls in the hope of stopping them temporarily. They provided a little water for themselves, from the cloaca. Sometimes an animal barely submerged in a four-inch, shallow bowl would undergo the expansion that usually precedes shedding and would virtually double up on itself in the cramped space. When noted, this was remedied out of sympathy and the animals went on to shed in larger quarters, but sometimes they were not moved, and shed in the small containers. Usually, however, shedding was observed among animals in the 6 by 9 inch or 6 by 7½ inch cylindrical battery jars which proved to be the most suitable containers for them. Here there was room for maximum expansion but at the same time some hope of retrieving the shed cells.

The water was usually changed several times a day to ensure freshness but it is questionable whether this is really necessary if the animals are stored in a cool place (15°–17° C.). One sturdy specimen was found shedding after at least two days in unchanged sea water at about 20° C. Pearse (1908) and others have shown that *Thyone* can endure a good deal of variation in the environment, such as higher salt concentration, dilution with fresh water, excessive heat (some survived more than two hours' exposure to as high as 37° C.), or exposure to air. It is unlikely, then, that large quantities of sea water are essential to shedding. However, it is probably desirable for storage, since animals stored in running sea water did shed, even many days after collection. It would be very hard to gather eggs or sperm shed at random in a large aquarium and, as a matter of fact, no sheddings were observed among stored animals. Possibly they escaped notice because of rapid dissipation of the gametes.

(6) *Freshness of Animals*

It is generally held that fresh, well-fed animals provide the best embryological material. In the case of *Thyone* there is one drawback to using animals just collected. Their habit of pumping mud through the gut soon clouds the water so that gametes would be very hard to see, if present. The waving of the tentacles provides an excellent stirring system which keeps the debris from settling. An animal which has been stored in a large aquarium, or in running sea water, overnight or longer, has got rid of much of this material and is an easier one in which to watch shedding. Spawning often does occur several days after collection and in a few cases animals which had been in the laboratory as long as nine and ten days were found shedding. Normal larvae developed from eggs shed two or three days after the parents were collected. The problem, then, is to keep the animals from shedding until after they have emptied their digestive tracts.

(7) *Temperature*

Warming. There is some evidence that temperature plays an important role in the spawning of *Thyone*. Several times when half of a group of animals was kept at room temperature (20°–22° C.) while the other half remained at the temperature of the sea water (16.5°–18° C.), a number of sheddings occurred among the warmed animals, none among those kept cool. Precise tests of this were not feasible this year because of the increasing number of spent animals among those collected. The most suggestive results were obtained with 34 specimens collected on or before June 21st. At 3:35 P.M. on that date, 17 animals, group A, were placed in water at room temperature while 17 others, group B, were left in the running sea water. Five and a half hours later the water was changed on group A, some of the animals being put into fresh cold sea water, the rest into fresh warm sea water. Within a half hour shedding began in the group returned to cool water, and an hour and a half later it began in the group continued warm. In all, there were 12 out of a possible 17 sheddings. Meanwhile the animals of group B, kept cool while the others were being warmed, were placed in containers of fresh sea water and under the same lighting conditions as group A (typical overhead electric light of this laboratory). Part were put into cool water and the others into water at 20° C. There was no shedding at all during the next three hours or indeed overnight after the animals had been returned to the cold water table. Yet subsequent examinations showed that at least some of these animals could have shed, though some were already spent. The warming, if this experiment is indicative, should be of longer duration than three hours and more in the vicinity of five and a half, or more, hours.

Further support for the belief that warming induces shedding comes from the totals of the whole season's studies: No sheddings were ever observed in animals which had not been warmed for some time beforehand. Unfortunately, however, many animals which were warmed failed to shed, and others did not shed the first time they were warmed, but only after warmings on several days or after staying warm continuously for several days. Perhaps results such as these may be attributed to the waning season, or to the possibility that animals kept in confinement for many days, especially after being warmed and cooled spasmodically, may react with less predictability than fresher ones. Additional exploration of this aspect of shedding is certainly needed.

Cooling after warming. A change to cool water following a period of warming often seemed to stimulate shedding, but since most of these cases occurred at the commonest shedding time, from 8:00 to 11:00 P.M., time of day could not be ruled out as part of the stimulus. The best favoring evidence was this: A group of animals which had been in the supply house for at least a day was brought to the laboratory at 1:20 P.M. on June 22nd. They were in water of 17.8° C. which rose to 18.8° C. by 2:50 P.M. At that time all the jars of animals were placed in a cooler in which the temperature was about 19° C. but was gradually descending. At 5:45 P.M. the temperature had reached 16.5° C. and a number of animals of both sexes were shedding. Warming followed by cooling had certainly occurred but the exact time of the two periods is not known. There were also other factors. The cooler was entirely dark while housing the animals and it was vibrating vigorously. The matter of light has been discussed above and may be dismissed here. In view of the animals' normal reaction to jarring (Pearse, 1908) it seems as if the shedding may have occurred in spite of this obstacle. The time at which the shedding took place is especially interesting. It was much earlier than usual. Could it be that the treatment had overcome another possible obstacle, namely, time of day?

Continued cooling. Just as warming, or warming followed by cooling, may possibly stimulate shedding, so continued cooling appears to prevent it from taking place. If freshly collected animals are not wanted for immediate use they should be kept as cool as the water they came from, that is, no warmer than 15°–17° C., to discourage shedding. On the other hand, once an animal has started to shed, sudden cooling does not seem to stop it.

(8) *Other Factors*

A few attempts were made to stimulate shedding by other, more artificial, means. External mechanical stimuli such as jarring, squeezing or pricking merely resulted in quick contraction with tentacle withdrawal, wholly unfavorable to shedding. More drastic treatment, like cutting, either caused contraction as above or evisceration which makes shedding quite impossible. Injections into the body cavity can be effected without harming the animal, the amount being gauged by the size of the body. Pearse (1909), studying the physiological effects of various substances, suggested that some of his results were influenced by the fact that the animals were spawning. Conversely, it might be that some of the shedding was induced by his treatment. For instance, he found that sodium chloride, in 10% solution, resulted in "feeding movements" and otherwise caused no harm to the animals. The same reaction was obtained this year, using 0.5 to 1.5 cc. of 10% NaCl. It seemed as if the animals might be just about to shed, but no shedding ensued. This treatment should be attempted again at the height of the spawning season. Palmer (1937) found potassium chloride a shedding stimulant in sea urchins. This was not explored carefully in *Thyone* and should also be repeated earlier in the season. However, injections of KCl did not seem to be followed by the "feeding movements" that followed the NaCl injections and, moreover, some animals responded to it by eviscerating.

Summary

1. Spawning occurred in the sea-cucumber, *Thyone briareus,* during the month of June, 1948. The phenomenon appeared to wane as the month progressed. Probably early June and possibly even earlier months are the best time to find high percentages of ripe germ cells.

2. The process of shedding is described, together with various details concerning the handling of shedding animals and their gametes.

3. The following possible factors in shedding are discussed: Time of day, amount of light, presence of a shedding male to induce shedding by the female, size, quantity of sea water, freshness of animals, temperature, etc. It is shown that the first three are not essential prerequisites for shedding.

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